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*Research Article*

# Green Synthesized Calcium Oxide Nanoparticles (CaO NPs) Using Leaves Aqueous Extract of *Moringa oleifera* and Evaluation of Their Antibacterial Activities

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Calcium oxide nanoparticles (CaO NPs) have unique catalytic and biological properties; their activities are highly influenced by their morphology; as a result, these characteristics are most needed for various applications in several fields, including material science, environmental science, and medicinal science. The primary motivation for synthesizing CaO NPs using a biological method is to suppress the usage of hazardous chemicals used in making its process, which will be more cost-effective and ecologically profitable. However, due to the complexity of the biological extracts employed in chemical processes, large-scale manufacturing of nanoparticles via the green synthesis approach remains a significant problem. As a result, the production of CaO NPs utilizing *Moringa oleifera* plant leaves aqueous extract as an alternative biological agent for capping, stabilizing, and reducing agents due to rich phytochemical parameters in synthesis was investigated in this study. The structural characterization of the CaO NPs obtained by using UV-Vis, FTIR, XRD, and SEM-EDS indicates the presence of purity and primarily aggregated spherical nanosized material with an average size of 32.08 nm observed. The XRD study revealed that heat annealing increased the size of the crystallites, favoring monocrystalline. Finally, these findings, together with the cheap cost of synthesizing the plant-mediated CaO NPs produced, show good antimicrobial (gram-positive) activities.

## 1. Introduction

Technology must be miniaturized to nanoscale size materials to function in the twentieth century. Nanostructures are a scientific discipline that involves manipulating materials on a nanoscale level. Nanomaterials act like atoms because they have a larger surface area, enormous surface potential, and unique surface properties [1]. These nanosized materials

Figure 1: *Moringa oleifera* plant.

exhibit high surface by volume ratios compared to bulk materials. The physical characteristics of larger particles are stable, have less surface volume ratio, and limit their applicability in many disciplines. Because of their size, shape, and morphology, bulk materials exhibit improved and unique characteristics when manipulated at the nanoscale level [2]. As an individual, they are helpful in various disciplines, including material science [3], photochemistry, medicine [4], and solar energy [5]. Calcium oxide (CaO) finds wide used in cosmetics, medicine, waste remediation, destructive adsorbent, and catalyst [1]. Chemical precipitation, hydrothermal, microemulsion, sol-gel, gas phase, microwave synthesis, and electrochemical methods have been described for CaO NPs synthesized and for other nanomaterials such as NiO, FeO, ZnO, Ag, and Au. Green method is significant for cost-effective, harmless, and eco-friendly than any other methods like chemical and physical [6, 7]. According to the literature, some of the phytoremediation notions found in the plant extract may be related to nanomaterial production's chemical mechanism [8, 9]. Metal/metal oxide nanoparticles (NPs) are considered the best way for future generations to use everywhere within clinical care, consumer items, and other industrial applications [6, 10]. The adsorption of $CO_2$, biomedical applications, gas sensing, and photocatalytic activity of CaO NPs have also gotten much attention [11].

*Moringa oleifera* plant, as shown in Figure 1, is a drumstick evergreen or deciduous tree of a single genus in the *Moringaceae* family; because of its numerous nutritional, medicinal, and industrial benefits [12, 13], it is regarded as a "wonder tree." It is a tiny, fast-growing ornamental tree found throughout Africa and Asia's tropical areas. It is primarily appreciated for its edible parts as fruits, leaves oil, leaves, and flowers, and it is widely utilized in traditional medicine [14]. According to a literature survey, the leaves have a high nutritional content, including vitamins, minerals, and amino acids used as an alternate source for dietary supplements [15] and growth boosters [16, 17]. Because of their high coagulation properties, the *M. oleifera* seeds powder has traditionally been used for purification of water in many rural regions [18]; in coagulation studies, a slight reduction in the total bacterial count of the filtered water was found, with antibacterial and antioxidant effects, indicating that the seeds may contain antibiotic compounds [19]. *Moringa* is said to offer a variety of therapeutic properties [12, 14]. Thus, the *M. oleifera tree* parts are used in traditional medicine to cure diarrhea, hypertension, and folk medicines. As a result, herbal plants used in medicine, also known as phytomedicine, are still reliable [12]. Plant components function as cardiac stimulants and anticancer [20], antiulcer, antipyretic, and anti-inflammatory [15]. The *M. oleifera* Plant also has anticancer, antimicrobial, antidiabetic, antibiotic, antihypertensive, antioxidant, hepatoprotective, and cholesterol-lowering effects [14, 21, 22].

Due to the more effective and environmentally friendly approach to the biosynthesis of CaO NPs, the utilization of

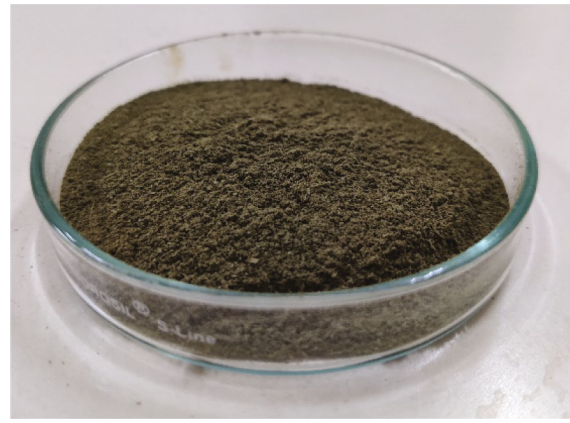


Figure 2: *Moringa oleifera* leaf powder.


(a)

(b)

Figure 3: (a) *Moringa oleifera* leaves powder extract and its basic solution. (b) Yellow-colored precipitate of MO-CaO NPs.

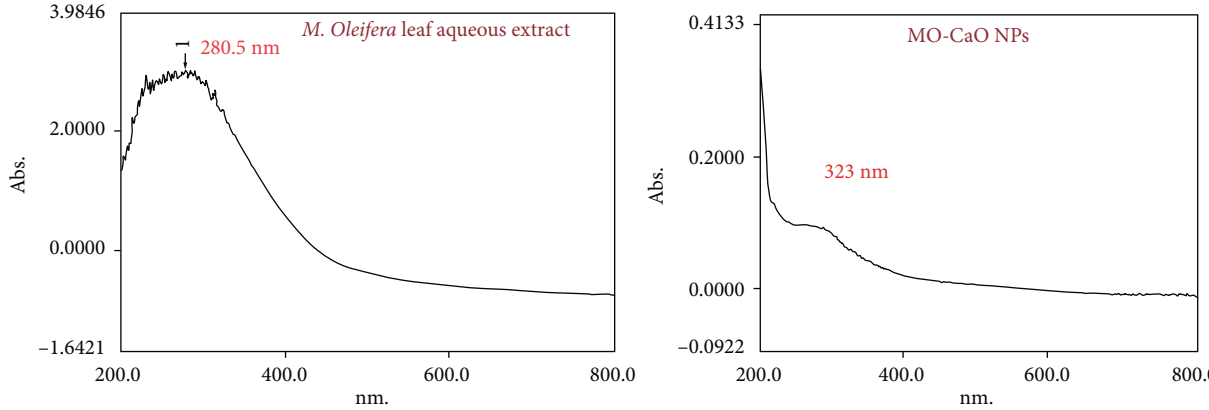



Figure 4: UV spectra of *M. oleifera* leaf aqueous extract and MO-CaO NPs.

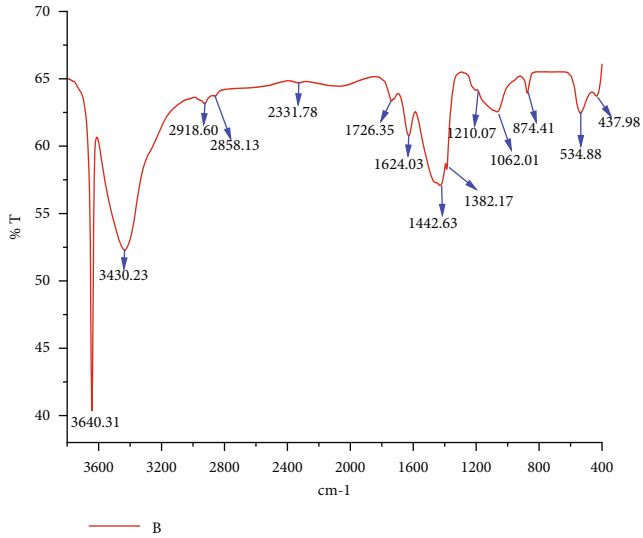



Figure 5: FTIR spectrum of MO-CaO NPs.

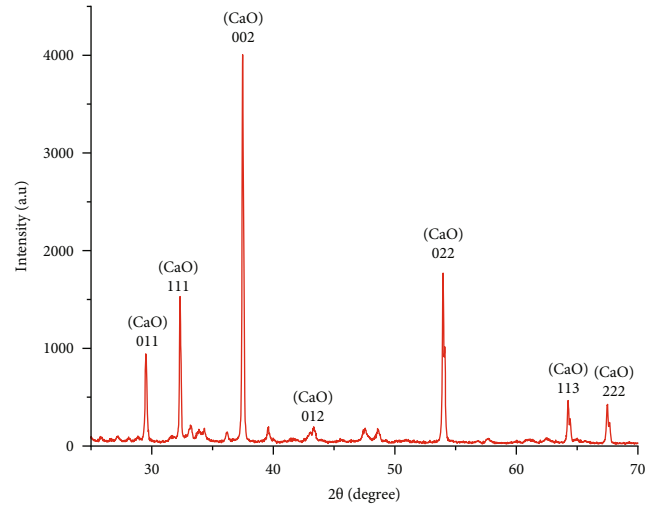



Figure 6: XRD pattern of MO-CaO NPs.

Table 1: XRD data.

| No. | 2$\theta$ | $\Theta$ | d-Spacing (A°) | Intensity (a.u) | (hkl) |
|---|---|---|---|---|---|
| 1 | 29.596 | 14.798 | 3.015 | 921.858 | 011 |
| 2 | 32.216 | 16.108 | 2.775 | 1302.459 | 111 |
| 3 | 37.348 | 18.674 | 2.405 | 3171.038 | 002 |
| 4 | 42.812 | 21.406 | 2.109 | 436.0656 | 012 |
| 5 | 53.829 | 26.914 | 1.701 | 1424.864 | 022 |
| 6 | 64.091 | 32.045 | 1.451 | 432.2400 | 113 |
| 7 | 67.335 | 33.667 | 1.388 | 416.9390 | 222 |

plant leaves aqueous extracts will be a worthy scientific challenge [4, 23, 24]. It is long been recognized that phytochemicals present in a plant material can function as a biological reduction on metal and metal oxide production [25, 26], with flavonoid compounds being one of the most influential families of secondary metabolites in plant tissues for metal ion reduction [27]. Depending on the kind of plant, most flavonoid compounds include ascorbic acid, phenolics, and carotenoids [16, 22]. "*M. oleifera* leaf has been reported to be a rich source of beta-carotene, protein, vitamin C, carotenoids, polyphenols, phenolic acids, flavonoids, alkaloids, glucosinolates, isothiocyanates, tannins, saponins, calcium, and potassium, as well as a good source of natural antioxidants, and thus improve the shelf life of fat-containing foods due to various antioxidant compounds such as ascorbic acid, flavonoids, phenolics, and carotenoids" [12, 28]. In recent research, *Moringa* leaf powder aqueous extracts have been used to make nanoparticles [16]. This is the first time a shrub has been used for green nanoparticle deposition. We perform work for the biosynthesis of CaO nanoparticles with the employment of *Moringa oleifera* leaves aqueous extract secondary metabolite compounds and its study regarding antibacterial activity because there is still a lack of scientific reports on using the extract of *Moringa oleifera* (MO) leaves as a biologically reductive and capping agent on the biosynthesis of MO-CaO nanoparticles [16]. Furthermore, the antibacterial activity outline of nanomaterial was investigated in contrast to bacterial fears, with the final result revealing that Gram-positive (G+) bacteria (*Escherichia coli*) are more vulnerable than Gram-negative (G-) bacteria at various concentrations.

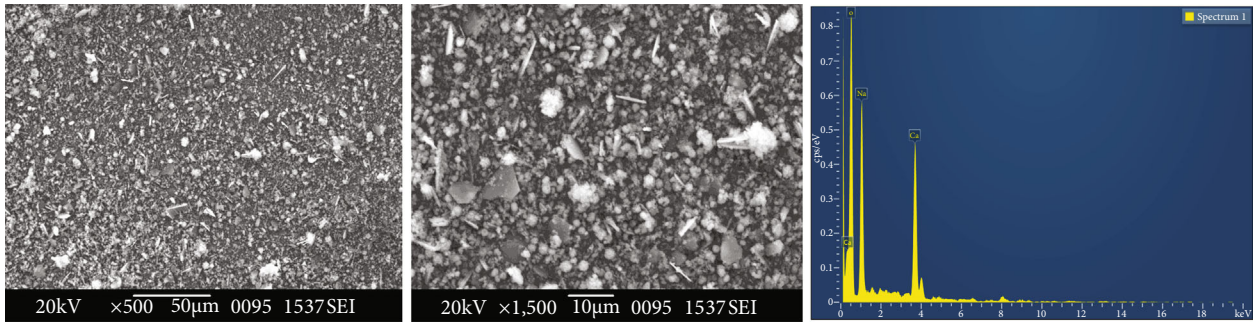



Figure 7: SEM images and EDS of MO-CaO NPs.

Table 2: Ca and O elements composition in the synthesized CaO nanoparticles.

| Element | Line type | Wt% | Atomic % |
|---|---|---|---|
| O | K series | 59.81 | 72.22 |
| Na | K series | 23.45 | 19.71 |
| Ca | K series | 16.74 | 8.07 |
| Total: | | 100 | 100 |

## 2. Materials and Methods

*2.1. Materials.* Analytical grades with 98% of purity of calcium nitrate Ca $(NO_3)_2 6H_2O$ and sodium hydroxide (NaOH) and deionized water grade-I (Extra pure) Charco Chemicals. *M. oleifera* leaves are collected through the local farm in Nashik, Pimpalgaon (B), Maharashtra, India.

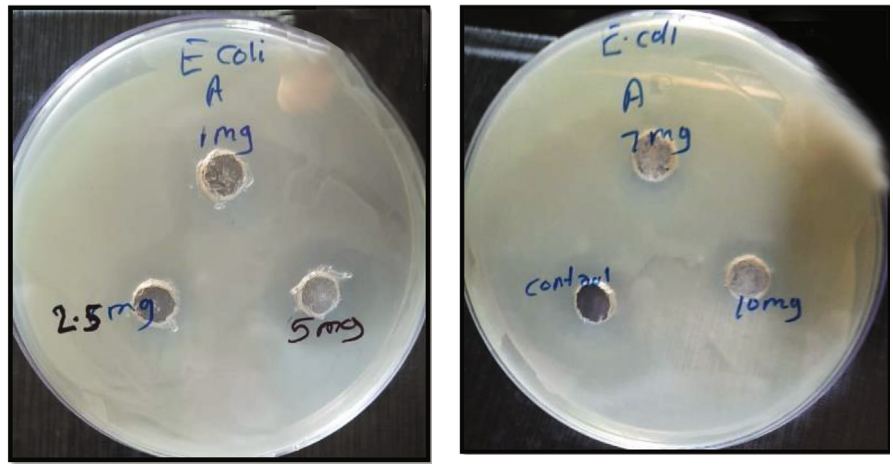
*2.2. Preparation of Moringa oleifera Plant Leaves Powder.* Fresh mature *Moringa* leaves were collected during the daytime from the local farm, removed the leaves and discarded the stems and stalks, and then thoroughly were washed with distilled water and followed with deionized water to remove the various impurities such as mud, dust particles, and unwanted material. The MO leaves were allowed to dry in the oven at 80°C for 2 hrs after drying and made powder using mortar and pestle, and leaves powder, as shown in Figure 2, was taken for MO-CaO NPs synthesis purposes.

*2.3. Synthesis of Calcium Oxide Nanoparticles Using M. oleifera Leaves (MO-CaO NPs).* The 2 g MO leaves powder was boiled in deionized water for 25 min at 60°C. A light brown colored solution in Figure 3 was formed during the boiling and cooled at room temperature (RT). After that, the brown-colored aqueous extract was filtered (Whatman No. 1), and the filtrate (stock solution) was stored in the refrigerator (2°C to 4°C) for further work. 20 mL MO plant aqueous extract was boiled at 50°C to 60°C using a magnetic stirrer. The temperature of the solution was reached at 50°C to 60°C; 2 g of calcium nitrate $(Ca(NO_3)_2\ H_2O)$ was added to maintain the basic nature of the aqueous extract solution by using a few drops of sodium hydroxide (2 M NaOH) solution [29] as shown in Figure 3(a). Then the mixture was boiled until it became a yellow-colored paste (Figure 3(b)) and filtered through filter paper, and the precipitate was transferred to a crucible and heated in a muffle furnace up to 400°C for 3 hrs to obtain the white powder; it is the MO-CaO NPs which were identified by the characterizations and used for various applications.

## 3. Results and Discussion

*3.1. UV-Vis Absorption Spectroscopy Analysis.* The absorption spectra of *M. oleifera* leaf aqueous extract and MO-CaO NPs with an absorption peak of about 280.5 nm and 323 nm, respectively, are shown in Figure 4. The leaf extracts bump at 250 nm to 300 nm; it could be the presence of polyphenolic bioactive compounds responsible for reducing calcium ions to CaO NPs [30]. The UV-Vis spectra of MO-CaO were taken in the solid-state, with the substantial excitation binding energy at 25°C; MO-CaO NPs show exciton absorption at 323 nm. Demonstrating UV-Vis of MO-CaO NPs, it can be seen that the nanomaterial has a good absorption capacity in the visible region [11].

*3.1.1. Vibrational Properties.* The aqueous extract of *MO* contains a lot of phytochemicals such as alkaloids, glycosides, phenols, tannins, saponins, glucosinolate, and flavonoids. These major bioactive components will act as capping and stabilizing agents and can be reduced from calcium metal salt $(Ca^{2+})$ precursors to CaO NPs. To further affirm the formation of the CaO NPs crystal structure, FT-IR spectroscopy is shown in Figure 5. The strong IR band located at 534 $cm^{-1}$ attributed to fundamental vibrations of CaO, confirming the formation of CaO NPs. The broad peak observed at 1062 $cm^{-1}$ corresponds to the C-O stretching frequency of phytochemicals in the *Moringa oleifera* aqueous leaves extract. The peaks around 1382 $cm^{-1}$ correspond to the O-H bend of flavonoids and polyphenols, confirming the presence of an aromatic group. The absorption peak appearing around 1442 and 3430 $cm^{-1}$ could be correlated to adsorbed water's bending and stretching vibrations bands and the residual -OH group. The bioactive/biomolecules in leaf extract of *Moringa oleifera* are responsible for the capping, reducing, and stabilizing CaO NPs. To identify the nature of the MO extract, which was responsible for the reduction of calcium ions to form the CaO NPs, the aqueous extract of MO were investigated [30]; the major bioactive compounds, as shown in Scheme 1 in *Moringa oleifera* leaf aqueous extract, may be responsible for capping and reducing the nature of the CaO NPs [30].




(a) (b)

Figure 8: The antibacterial activity of plant-mediated CaO NPs (a) and (b) *Escherichia coli* at various concentrations.

*3.2. FT-IR Analysis.* For the MO-CaO NPs and *M. oleifera* leaf aqueous extract, IR measurements were performed at room temperature using the KBr technique in the wavenumber range (400 to 4000 $cm^{-1}$). The CaO bond bending vibration appears as a peak in the low energy area at 437.98 $cm^{-1}$. Ca-oxygen is responsible for the zone between (400 and 600) $cm^{-1}$. The stretching vibration of the -OH group is responsible for the prominent peak in the higher energy area at 3600–3450 $cm^{-1}$. At roughly 2933–2915 $cm^{-1}$, the -CH stretching vibration band appears, indicating the existence of an alkanes group. The amide I and amide III sections of proteins/enzymes are responsible for the peaks around 1620–1655 and 1150-1390 $cm^{-1}$. The presence of functional groups of alcohols, and a carboxylic acid, may be seen in the influential bands detected at 1120–1065 $cm^{-1}$ C–O stretching vibration. The absorption bands at 3430, 2918, 1624, 1442, and 1062 $cm^{-1}$ in the FT–IR spectra indicate the structure of *M. oleifera* in which high phytochemicals parameters caused these peaks, as shown in Figure 5. As a result, water-soluble phenolic acid and flavonoid components are essential in the bioreduction process. The interactions between reducing phenolic acids such as ascorbic, cardiac glycoside, gallic acid, and calcium ions can lead to MO-CaO NPs. The mechanism of CaO NPs stabilization by *M. oleifera* leaf extract ascorbic acids and green production of CaO NPs might entail the reduction of calcium nitrate ions, which can form intermediate complexes with phenolic -OH groups found in hydrolyzable tannins, which are then oxidized to quinine forms, resulting in calcium reduction to CaO NPs. However, the process is yet unknown and has to be investigated further.

*3.3. X-Ray Diffraction Microscopy (XRD).* The XRD patterns show perceptible peaks of CaO NPs at 29.59°, 32.22°, 37.35°, 42.81°, 53.83°, 64.09°, and 67.34°, corresponding to (011), (111), (002), (012), (022), (113), and (222) planes of cubic system CaO NPs as shown in Table 1. The plane values of XRD patterns agree with CaO (lime, syn, JCPDS card No. 00-004-0777) with cubic crystal system and lattice parameters ($a = 4.8152$ A°). All recorded peak intensities were characteristics of the cubic structure; the relatively high intensity of the (002) peak indicates anisotropic growth and implies a preferred orientation of the crystallites, as shown in Figure 6. The stiff and narrow diffraction peaks suggest that the product is excellent crystalline. There is no shift in the diffraction peaks, and other crystalline impurities are not observable.

*3.3.1. Calculation of d-Spacing between Calcium Oxide Nanoparticles.* It can be calculated by using Bragg's equation:

$$n\lambda = 2d \operatorname{Sin} \theta \tag{1}$$

where $\lambda = 1.54$ A° wavelength of X-rays, $n$ is the order of diffraction ($n = 1$), $d$ is the distance between adjacent CaO layers, and $\theta$ is the diffraction angle.

d-Spacing is calculated by using above equation:

$$d = \frac{n\lambda}{2x \sin \theta} \tag{2}$$

Crystal planes for the cubic system:

$$d_{\mathrm{hkl}} = \frac{a}{\sqrt{h2 + k2 + l2}}$$
$$a = b = c = 4.8152 \text{ Å} \tag{3}$$

According to the Scherrer equation,

$$D = \frac{K\lambda}{\beta COS\theta} (D \leq 200 \text{ nm}) \tag{4}$$

where $D$ is the average crystallite size/diameter (particle size); $K$ is the Scherrer constant, 0.68 to 2.08, 0.94 for spherical crystallites with cubic symmetry; $\beta = \beta' \pi/180$, broadening at FWHM in radians; $\beta'$ is the full width at half maximum; $\lambda$ is the X-rays wavelength ($CuK_\alpha = 1.5408$ Å); and $\theta$ is the Glancing angle in degrees half of $2\theta$.

Using the above Scherrer equation, the mean or average particle size of the plant-mediated CaO NPs is 32.08 nm.

Table 3: Concentration dependence of the antibacterial activity of MO-CaO NPs against *Escherichia coli and Staphylococcus aureus* (ND: not detected).

| Compound | Concentration | Gram (+) bacteria *Escherichia coli* | Gram (-) bacteria *Staphylococcus aureus* |
|---|---|---|---|
| MO-CaO NPs | 1.0 mg/ml | 19 mm | ND |
| | 2.5 mg/ml | 20 mm | ND |
| | 5.0 mg/ml | 22 mm | ND |
| | 7.5 mg/ml | 23 mm | 9 mm |
| | 10.0 mg/ml | 24 mm | 11 mm |

*3.4. Scanning Electron Microscopy and Energy Dispersive X-Ray Analysis (SEM-EDS).* The sample's SEM studies in Figure 7 show the synthesized MO-CaO NPs. The images depicted both individual CaO NPs and the number of aggregates. The picture shows that the particles are round, granular, and nanosized. The size and form of the MO-CaO NPs are visible in the SEM images. NPs were spherical with a group of aggregated particles; according to XRD analysis, the average particle sizes are within 30–40 nm, as shown in Figure 6. To confirm the purity of the MO-CaO, the energy dispersive X-ray analysis was done. EDS spectrum clearly shows that the synthesized material is highly pure, which shows peaks due to Ca, O, and Na elements as shown in Figure 7 and Table 2, and the presence of Na may be due to the basic solution of MO plant leaves aqueous extract. The FTIR analysis shows that Ca and O atoms produce weak and strong bonding peaks due to macromolecules such as alcoholic or phenolic compounds; weak peaks are detected from the elements S, K, C, Na, Ca, and O [5].

*3.4.1. Antibacterial Activity.* According to the minimal inhibitory concentration (MIC) method, *Escherichia coli and Staphylococcus aureus* were used as microorganisms in this study. Stock solutions of synthesized compounds were prepared in DMSO. Cultures were incubated in nutrient broth for 24 hrs at 37°C at 160 rpm. The turbidity of bacterial suspension was adjusted at a concentration of approximately $10^6$ cells/ml. The microorganisms grown were spread on the nutrient agar medium plate, and then a well was prepared with the help of a good borer. Then 100 $\mu$l of a synthesized compound of various concentrations was added to each well, as shown in Figure 8. After that, all plates were incubated at 37°C for 24 hrs in the incubator. The minimum inhibitor concentration was taken as the MIC value, at which no growth was observed.

## 4. Conclusion

Leaf powder extracts of the *Moringa oleifera* plant act as capping and reducing agents due to phytochemical parameters used to make CaO nanoparticles for this study. Still, the reaction mechanism and specific bioactive groups are unknown and must be investigated further. The use of nanoparticles made from leaf extracts is a promising alternative to the more conservative chemical method. Ultraviolet visible (UV-Vis), Fourier transform infrared resonance (FTIR), X-ray diffraction (XRD), and scanning electron microscopy-energy dispersive X-ray analysis (SEM-EDS) were used to examine the plant-mediated CaO NPs. Furthermore, using the minimal inhibitory concentration (MIC) method approach, this work investigated the antibacterial activities of the produced CaO nanoparticles in contrast to scientific and traditional concerns. Finally, CaO nanoparticles synthesized from *M. oleifera* leaf powder aqueous extract exhibited promising results in Gram-positive bacterial strains, as shown in Table 3 with severe inhibition areas of 19 mm, 20 mm, 22 mm, 23 mm, and 24 mm at various concentrations when employing a consistent quantity of CaO NPs.

## Data Availability

All data used to support the findings of this study are included within the article.

## Conflicts of Interest

The authors declare that they have no conflict of interest.

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